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## COMPLEMENT FIXATION IN INTESTINAL PARASITISM OF DOGS \*

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Infestation of the intestines with the commoner varieties of parasites is generally regarded as exercising no particular harm on the host, except to produce such general disturbances as digestive derangements or anemia, the latter being ascribed to the loss of blood through blood-sucking parasites and to the absorption of hemotoxic substances. Examples are not wanting, however, both among men, and among animals—notably the dog and the horse—of blood changes and many symptoms of disease which may be ascribed to the production by the parasites of toxic substances, capable of being absorbed and exercising harmful effects.

The object of our study was to determine by means of a complement-fixation technic whether absorption of foreign substances with the production of antibodies occurred in dogs infested with the common varieties of intestinal parasites. That production of antibodies may result from superficial infections is shown by the immunologic studies in parasitic diseases of the skin by Kolmer and Strickler,<sup>1</sup> in which it was found that in ring-worm of the scalp and favus, by means of a complement-fixation technic specific antibodies might be detected in the blood serum in a large majority of diseased individuals. In ring-worm particularly, the fungus seldom penetrates to the deeper layers of the epidermis and rarely to the corium; hence, it may be assumed that soluble toxic substances are produced by the fungus, which being absorbed cause the production of antibodies. The encouraging results of this work induced us to undertake a similar study of intestinal parasitism, dogs being selected on account of the frequency with which they are infested and the means offered for controlling the results by frequent examinations of the feces and by autopsies.

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<sup>1</sup> Jour. Am. Med. Assn., 1915, 64, p. 800.

## HISTORICAL REVIEW

Several investigators have employed a complement-fixation technic in the diagnosis of echinococcus disease of the liver. Ghedini<sup>2</sup> found this test of value, and he also reports specific reactions with the sera of persons infested with *Ankylostoma duodenales* and *Ascaris lumbricoides*.<sup>3</sup> Weinberg,<sup>4</sup> Jiani,<sup>5</sup> Israel,<sup>6</sup> and Henius<sup>7</sup> report favorable and specific reactions in echinococcus disease with aqueous or alcoholic extracts of cyst fluid or both. Kurt Meyer<sup>8</sup> found these reactions non-specific, in that the serum of a person infested with echinococcus showed complement-absorption with antigens of *Tenia solium* and *T. saginata*, and vice versa. Branes<sup>9</sup> found that alcoholic extracts of echinococcus cyst fluid showed complement-absorption with the syphilis antibody as well as with that of echinococcus disease, and this observation has been generally confirmed. Thomsen and Magnusson<sup>10</sup> in a study of 12 cases of echinococcus disease found that the sera of 10 reacted positively; the sera of 55 control cases (32 of which reacted positively to the Wassermann reaction) all were negative except one. These authors also report favorably on the specificity of the reaction; the sera of 10 persons infected with *Tenia saginata*, of 2 with *T. solium*, and of 1 with *Bothriocephalus latus*, all were negative with echinococcus antigen.

In so far as echinococcus disease of the liver is concerned, a review of the literature shows a general consensus of opinion that antibodies are present in the sera of the majority of diseased persons and animals and that these may be detected by means of a complement-fixation test. There is, however, a division of opinion in regard to the specificity of the reaction and its practical value in diagnosis.

Much less work has been reported on complement fixation with sera of persons infested with such parasites as *Tenia saginata*, *Ascaris lumbricoides*, etc. Of interest in this connection, as showing the probability of antibody-formation in infestations with these parasites and in protozoal infections in general, are the reports of Rubinstein and Julien,<sup>11</sup> Manoileff,<sup>12</sup> Gozony,<sup>13</sup> and Hindle and Gozony,<sup>14</sup> who found ferments in the sera of individuals, as determined by the methods of Abderhalden, using as substrates preparations of *T. solium* and *T. saginata*, *Ascaris lumbricoides*, etc.

## MATERIALS AND METHODS OF STUDY

*Antigens.*—The following extracts of parasites, secured from dogs at autopsies, were prepared:

1. Salt-solution extract of *Tenia serrata*.
2. Alcoholic extract of *T. serrata*.

<sup>2</sup> Gaz. degli Ospedali e delle Cliniche, 1906, 27, p. 1616; 1907, 28, p. 53.

<sup>3</sup> Ibid., 1907, 28, p. 476.

<sup>4</sup> Ann. de l'Inst. Pasteur, 1909, 23, p. 472 (in which references are given to his previous work in this field).

<sup>5</sup> Wien. klin. Wchnschr., 1909, 22, p. 1439 (in which the author gives a good bibliography of earlier literature).

<sup>6</sup> Ztschr. f. Hyg. u. Infektionskrankh., 1910, 66, p. 487.

<sup>7</sup> Deutsch. med. Wchnschr., 1911, 37, p. 1212.

<sup>8</sup> Berl. klin. Wchnschr., 1910, 47, p. 1316.

<sup>9</sup> München. med. Wchnschr., 1911, 58, p. 1073.

<sup>10</sup> Berl. klin. Wchnschr., 1912, 49, p. 1183.

<sup>11</sup> Compt. rend. Soc. de biol., 1913, 75, p. 180. Abstract by Graltz, Ztschr. f. Immunitätsf. R., 1913, 8, p. 63.

<sup>12</sup> Wien. klin. Wchnschr., 1914, 27, p. 269.

<sup>13</sup> Centralbl. f. Bakteriöl., I, O., 1914, 73, p. 345.

<sup>14</sup> Parasitology, 1914, 7, p. 228.

3. Salt-solution extract of *Dipylidium caninum*.
4. Alcoholic extract of *Dipylidium caninum*.
5. Salt-solution extract of *Ascaris canis*.
6. Alcoholic extract of *Ascaris canis*.
7. Salt-solution extract of *Trichocephalus dispar* (whip worm).
8. Salt-solution extract of *Strongylus gigas*.
9. Alcoholic extract of *T. saginata*.

Antigen 9 was prepared from a parasite from a human host and is included in this series for the purpose of studying the specificity of the reactions with parasites from dogs.

*Strongylus gigas* is found in the pelvis of the kidney, and while, accordingly, it is not an intestinal parasite or a common type of infestation, we thought it would be of interest to include this antigen in our series.

These parasites, representing the commoner varieties to be found in dogs in this district, sufficed for the purposes of this work; namely, to ascertain whether their presence in an animal was followed by the production of antibodies that could be detected in the serum by means of a complement-fixation test.

In the preparation of each salt-solution antigen the fresh parasites were washed several times, and 4 grams thoroughly ground and macerated with sand and powdered glass and then suspended in 100 c.c. of sterile salt solution containing 0.5% phenol. This mixture was shaken mechanically for 24 hours, incubated at 37 C. for several days, and then filtered and stored in the refrigerator.

The alcoholic extracts were prepared in the same manner with the use of absolute ethyl alcohol, except that extraction in the incubator was continued for a longer period of time.

Antigens prepared in this manner contained a portion of the protein and other constituents of the parasite, as well as endotoxic substances, and generally proved satisfactory in the titrations and complement-fixation tests.

All antigens were diluted with salt solution and titrated at frequent intervals, usually just before the complement-fixation tests, and used in amounts corresponding to one-quarter of their anticomplementary doses. The salt-solution extracts were found to vary in their anticomplementary units to such an extent as to require titration before every experiment; the alcoholic extracts were more stable.

Table 1 shows the method of titration and the doses employed, and is representative of these titrations.

*Hemolytic System.*—The antisheep hemolytic system was employed. Complement was furnished by the mixed sera of 2 or more guinea-pigs, and was used in dose of 0.05 c.c. (1 c.c. of 1:20 dilution). Antisheep hemolysin was titrated each time against this constant dose of complement and 1 c.c. of a 2.5% suspension of sheep cells, and was used in the antigen titrations and in complement-fixation tests in amounts equal to 2 hemolytic units.

In the anticomplementary titrations of the antigens increasing amounts of antigen (Table 1) were incubated with the complement for 1 hour at 37 C.; then 2 units of hemolysin and 1 c.c. of the corpuscle suspension were added, the whole mixed, re-incubated for 1 hour, and the results read.

In the complement-fixation tests fresh or inactivated sera in varying doses were incubated with the various antigens and complement for 1 hour; then 2 units of hemolysin and 1 c.c. of corpuscle suspension were added; after mixing and re-incubation for 1 hour or over, according to the hemolysis of the con-

trols, the results were either read at once, or the tubes were placed in a refrigerator and the reading made the following morning. As usual every serum and antigen and hemolytic system was controlled in each set of titrations and reactions.

*Sera.*—In all, the sera of 172 dogs were examined. As will be pointed out later in this paper, our greatest difficulty in this work was the tendency of dog serum to yield non-specific complement fixation. For this reason the sera were generally used in an active state or after inactivation at 62 C., and in various doses, ranging from 0.05 to 0.4 c.c. — usually 0.1 c.c. Blood was collected from each dog from the external jugular vein by means of sterile needles in sterile test tubes, and in those tests in which active serum was used, the reactions were conducted within 24 hours after the collection.

TABLE 1  
ANTICOMPLEMENTARY TITRATIONS OF ANTIGENS

Amount c.c.	Salt Solution Extract T. ser- rata	Alco- holic Extract T. ser- rata	Salt Solution Extract of Dip- tylidium caninum	Alco- holic Extract of Dip- tylidium caninum	Salt Solution Extract Ascaris canis	Alco- holic Extract Ascaris canis	Salt Solution Extract Tricho- cephalus dispar	Salt Solution Extract Strong- ylus gigas	Alco- holic Extract T. sagi- nata
	1:10	1:5	1:20	1:20	1:5	1:20	1:4	1:10	1:20
0.2	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis
0.4	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis
0.6	Marked hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Marked hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis
0.8	Slight hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis
1.0	No hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis	Marked hemol- ysis	Com- plete hemol- ysis	No hemol- ysis	Marked hemol- ysis	Com- plete hemol- ysis	Com- plete hemol- ysis
2.0	No hemol- ysis	Slight hemol- ysis	Slight hemol- ysis	Slight hemol- ysis	Marked hemol- ysis	No hemol- ysis	Slight hemol- ysis	Marked hemol- ysis	Marked hemol- ysis
Dose	0.1	0.4	0.4	0.25	0.5	0.1	0.25	0.5	0.5

*Method of Study.*—Each serum with few exceptions was tested with all the antigens. The results were checked up by examinations of the feces of the animals for ova and parasites and by autopsies. In our earlier work a few days or a week or more would elapse between serum tests and feces examinations, but because parasitic conditions may vary from time to time, feces were collected on the day of the collection of blood, or on the day following. Each specimen of feces was examined macroscopically and microscopically (in the latter case by the direct smear and centrifuge methods) for parasites and ova.

We wish to express our appreciation of the aid given us by Dr. Allen J. Smith and Dr. Damaso Rivas in the identification of ova and parasites in numerous instances.

#### DIFFICULTIES ENCOUNTERED IN THE WORK

In this study we were confronted with two main difficulties. The first was the tendency of dog serum to yield non-specific complement-fixation. We have particularly studied this phase of the subject, the results being given in a separate communication.<sup>15</sup> To overcome this non-specific factor, we have carefully titrated our antigens and used them in doses equal to one-quarter or less of their anticomplementary units; the hemolysin was used in amounts equal to, or double the hemolytic unit, and the sera were used in a fresh, active condition and again after inactivation, in doses varying from 0.05 to 0.4 c.c. Sera that proved anticomplementary in the complement-fixation tests were excluded; best results were secured with fresh and active sera in dose of 0.1 c.c., in which native dog complement and antishoop hemolysin aided in overcoming the antilytic and non-specific complement-fixation tendencies of the serum, or, after heating the sera at 62 C., instead of 56 C., for 30 minutes.

The second difficulty was our inability to ascertain how long a dog had been infested with a particular parasite, together with the difficulty of excluding infestations on the basis of single, or even multiple, negative examinations of the feces. As will be shown later, we have observed positive complement-fixation with various antigens among 28 dogs (Table 5) in the feces of which ova were not found. It is an open question whether one or two negative feces examinations should exclude intestinal parasitism, or, whether antibodies could not persist in the body fluids for some time after the expulsion of a parasite or parasites.

#### RESULTS

Serum tests and feces examinations were made of 110 dogs. The results of the feces examinations may be summarized as follows:

25 dogs, or 23%, showed the presence of the ova of *Ascaris canis*.

16 dogs, or 14.5%, showed the presence of ova *Ascaris canis* and *Trichocephalus dispar* (whip-worm).

7 dogs, or 6%, showed the presence of ova of *Tenia serrata*.

4 dogs, or 3.6%, showed the presence of the ova of *Dipylidium caninum*.

<sup>15</sup> Kolmer, Trist, and Heist: Jour. Infect. Dis., 1916, 18, p. 27.

22 dogs, or 20%, showed the presence of the ova of *Trichocephalus dispar*.

7 dogs, or 6%, showed the presence of the ova of *Trichocephalus dispar* and *Uncinaria canina* (*Ankylostoma caninum*).

29 dogs, or 26%, showed no ova or parasites in the feces.

Complement-fixation tests were made with 172 sera, including the 110 dogs which had had feces examinations. The sera of 62 additional dogs were examined, but the animals were removed before thorough examination of the feces could be made; accordingly, this group has not been included in an analysis of the results for this report.

The results of the complement-fixation tests did not tally with the results of the feces examinations. The important difference does not lie so much in the fact that dogs showed the ova of a certain parasite in the feces while their sera were negative with an antigen of that parasite in a complement-fixation test, as in the observation that the sera of many dogs reacted with the antigen of parasites the ova of which could not be found in the feces. As previously stated, we had no method of ascertaining whether or not these animals had been infested at an earlier period and the antibodies were still present in the blood, or whether the parasites were present in the intestinal tract with so few ova in the feces as to escape detection. The results, therefore, may be summarized in two ways: (1) Results of complement-fixation tests with the antigen or antigens corresponding to the ova found in the feces compared with the results of feces examinations; and (2) results in complement-fixation tests with the various antigens regardless of the results of feces examinations.

Tables 2 to 4, inclusive, show the results of complement-fixation tests with the sera of dogs the feces of which had been examined and classified according to the ova found; Table 5 shows the results of complement-fixation tests with the sera of dogs the feces examinations of which had yielded negative results.

The results of complement-fixation tests in *Ascaris canis* infestations, shown in Table 2, may be summarized as follows:

1. Of 36 dogs, the sera of 10, or about 30%, reacted positively with a salt-solution extract of *Ascaris canis*.
2. The sera of but 2 of these dogs, or about 5.5%, reacted positively with an alcoholic extract of *Ascaris canis*.
3. With an alcoholic extract of *Ascaris lumbricoides* positive reactions were observed with the sera of 11 dogs, or about 30%, and 5 of

TABLE 2  
RESULTS OF COMPLEMENT-FIXATION TESTS IN INFESTATIONS WITH ASCARIS CANIS AND  
TRICHOCEPHALUS DISPAR

Dog	Serum	Dose Serum, c.c.	Salt- Solution Ex- tract T. Ser- rata	Alco- holic Ex- tract T. Ser- rata	Salt- Solution Ex- tract Dip- ylid- ium Can- ni- num	Alco- holic Ex- tract Dip- ylid- ium Can- num	Salt- Solution Ex- tract As- caris Can- is	Alco- holic Ex- tract As- caris Can- is	Salt- Solution Ex- tract Tri- cho- ceph- alus Dis- par	Salt- Solution Ex- tract Stron- gylus Gigas	Alco- holic Ex- tract T. Sagi- nata	Ser- um Con- trol
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## Ova of Ascaris Canis in Feces

223A	Active	0.1	—	+	—	—	—	—	—	—	—	—
227A	Active	0.1	+++	++++	—	—	+++	—	++	+++	—	—
228A	Active	0.1	—	++++	—	—	+++	—	—	—	—	—
240A	Active	0.1	—	+	—	—	—	—	—	—	—	—
241A	Active	0.1	—	++++	—	—	—	—	—	—	—	—
245A	Active	0.1	—	+++	—	—	++	—	—	—	—	—
324	Active	0.1	—	++++	—	—	—	—	—	—	—	—
310	Active	0.1	—	—	—	—	—	—	—	—	—	—
321	Active	0.1	—	—	—	—	—	—	—	—	—	—
195A	Active	0.1	—	—	—	—	—	—	—	—	—	—
197A	Active	0.1	—	++++	—	—	—	—	—	—	—	—
201A	Active	0.1	—	—	—	—	—	—	—	—	—	—
204A	Active	0.1	—	++++	—	++	++++	+	—	+	—	—
207A	Active	0.1	—	++++	—	—	—	—	—	—	—	—
209A	Active	0.1	—	—	—	—	—	—	—	—	—	—
215A	Active	0.1	—	++++	—	—	++++	—	—	—	—	—
217A	Active	0.1	—	++++	—	+	++	—	—	—	—	—
251A	Active	0.1	—	—	—	—	—	—	—	—	—	—
449	Active	0.2	—	—	—	—	—	—	—	—	—	—
456	Active	0.2	—	—	—	—	—	—	—	—	—	—
457	Active	0.2	—	—	—	—	—	—	—	—	—	—
477	Active	0.2	—	—	—	—	—	—	—	—	—	—
385	Inactive	0.2	++++	++++	—	—	—	—	0	—	—	—
434	Inactive	0.2	—	—	—	—	—	—	—	—	—	—

## Ova of Ascaris Canis and Trichocephalus Dispar in Feces

228A	Active	0.1	+	++++	—	—	++++	+++	—	+++	—	—
230A	Active	0.1	—	+++	—	—	+	—	—	—	—	—
239A	Active	0.1	—	—	—	—	—	—	—	—	—	—
252A	Active	0.1	—	++++	—	—	—	—	—	—	—	—
307A	Active	0.1	—	—	—	—	—	—	—	—	—	—
198A	Active	0.1	—	—	—	—	—	—	0	—	—	—
191A	Active	0.1	—	++++	—	—	—	—	—	—	—	—
199A	Active	0.1	—	—	—	—	+	—	0	—	—	—
202A	Active	0.1	—	++++	—	—	—	—	0	—	—	—
407	Active	...	—	—	—	—	—	—	—	—	—	—
475	Active	0.1	—	—	—	—	±	—	—	—	—	—
427	Inactive	0.1	++++	++++	—	—	—	—	0	0	—	—

## KEY TO TABLES

- ++++ = 100% inhibition of hemolysis (strongly positive)  
 +++ = 75% inhibition of hemolysis (moderately positive).  
 ++ = 50% inhibition of hemolysis (weakly positive).  
 + = 25% inhibition of hemolysis (weakly positive).  
 — = Complete hemolysis (negative).



these reactions occurred with sera that were negative with the salt-solution and alcoholic extracts of *Ascaris canis*.

The results of complement-fixation tests in infestations with *Tenia serrata* and *Dipylidium caninum* are shown in Table 3. While the sera of a large number of dogs reacted positively with these antigens, particularly with the antigen of *T. serrata*, in surprisingly few were we able to find ova. The table includes only those dogs in the feces of which the ova or links were found.

TABLE 3  
RESULTS OF COMPLEMENT-FIXATION TESTS IN INFESTATIONS WITH *TENIA SERRATA* AND  
*DIPYLIDIUM CANINUM*

Dog	Serum	Dose Serum, c.c.	Salt-Solution Extract <i>T. Serrata</i>	Alcoholic Extract <i>T. Serrata</i>	Salt-Solution Extract <i>Dipylidium Caninum</i>	Alcoholic Extract <i>Dipylidium Caninum</i>	Salt-Solution Extract <i>Ascaris Canis</i>	Alcoholic Extract <i>Ascaris Canis</i>	Salt-Solution Extract <i>Trichocephalus Dispar</i>	Salt-Solution Extract <i>Strongylus Gigas</i>	Alcoholic Extract <i>T. Saginata</i>	Serum Control
Ova <i>Tenia Serrata</i> in Feces												
429	Inactive	0.1	+	+	—	—	—	—	—	—	—	—
434	Inactive	0.1	+	+	+	+	—	—	—	—	—	—
437	Active	0.1	—	—	—	—	0	—	—	—	—	—
320	Active	0.1	++	+++	—	—	—	—	—	—	—	—
459	Active	0.1	—	+++	—	—	—	—	±	—	—	—
Ova of <i>Dipylidium Caninum</i> in Feces												
519	Active	0.1	—	—	—	—	—	—	—	—	—	—
320	Active	0.1	++	+++	++	+++	—	—	—	—	—	—
208A	Active	0.1	++	++++	—	—	+	+++	0	++	++++	—
173A	Active	0.1	+	+++	++	++++	—	—	—	—	+++	—

The results shown in Table 3 may be summarized thus:

1. Of 5 dogs infested with *T. serrata*, the sera of 3, or 60%, reacted positively with a salt-solution extract of *T. serrata*, and 4, or 80%, with the alcoholic extract of this parasite.

2. Of 4 dogs infested with *Dipylidium caninum*, the sera of 3, or 75%, reacted positively with the salt-solution and alcoholic extracts of this parasite.

3. Of the sera of 5 dogs infested with *T. serrata*, none reacted positively with an alcoholic extract of *T. saginata*, whereas the sera of 4 dogs infested with *Dipylidium caninum* reacted with this antigen.

The results of complement-fixation tests in infestations with *Trichocephalus dispar* (whip-worm) are shown in Tables 2 and 4. They may be summarized as follows:

Of 34 dogs infested with the whip-worm, the sera of all were negative with a salt-solution extract of this parasite. On account of the small size of the worm we were unable to obtain a sufficient number for the preparation of an alcoholic extract.

TABLE 4

RESULTS OF COMPLEMENT-FIXATION TESTS IN INFESTATIONS WITH *TRICHOCEPHALUS DISPAR* AND *ANKYLOSTOMA CANINUM*

Dog	Serum	Dose Serum, c.c.	Salt-Solution Extract T. Ser-rata	Alco-holic Extract T. Ser-rata	Salt-Solution Extract Dip-ylid-ium Can-ninum	Alco-holic Extract Dip-ylid-ium Can-ninum	Salt-Solution Extract As-caris Can-is	Alco-holic Extract As-caris Can-is	Salt-Solution Extract Tri-cho-cephalus Dis-par	Salt-Solution Extract Stron-gylus Gigas	Alco-holic Extract T. Sagi-nata	Serum Control
Ova of <i>Trichocephalus Dispar</i> in Feces												
329	Active	0.1	—	++	—	—	—	—	—	—	—	—
330	Active	0.1	—	±	—	—	—	—	—	—	—	—
332	Active	0.1	—	—	—	—	—	—	—	—	—	—
335	Active	0.1	—	—	—	—	—	—	—	—	—	—
336	Active	0.1	—	—	—	—	—	—	—	—	—	—
318	Active	0.1	—	—	—	—	—	—	—	—	—	—
320	Active	0.1	—	—	—	—	—	—	—	—	—	—
319	Active	0.1	—	—	—	—	—	0	0	0	0	—
327	Active	0.1	—	—	—	—	—	—	—	—	—	—
194A	Active	0.1	—	++	—	—	—	—	—	—	—	—
216	Active	0.1	—	++++	—	—	—	—	—	—	—	—
400	Active	0.1	—	—	—	+	—	—	—	—	—	—
472	Active	0.1	—	—	—	+	—	—	—	—	—	—
453	Active	0.1	—	—	—	—	—	—	—	—	—	—
454	Active	0.1	—	++++	—	—	—	—	—	—	—	—
460	Active	0.1	—	—	—	—	—	—	—	—	—	—
462	Active	0.1	—	—	—	—	—	—	—	—	—	—
468	Active	0.1	—	—	—	—	—	—	—	—	—	—
470	Active	0.1	—	—	—	—	—	—	—	—	—	—
430	Active	0.1	—	—	—	—	—	—	—	—	—	—
Ova of <i>Trichocephalus Dispar</i> and <i>Ankylostoma Caninum</i> in Feces												
329	Active	0.1	—	—	—	—	—	—	—	—	—	—
418	Active	0.1	—	—	—	—	—	—	—	—	—	—
492	Active	0.1	+++	—	—	±	+	—	—	—	—	—
461	Active	0.2	—	—	—	—	—	—	—	—	—	—
458	Active	0.2	—	—	—	—	—	—	—	—	—	—
474	Active	0.2	—	—	—	—	—	—	—	—	—	—
428	Inactive	0.1	++++	++++	—	—	—	—	—	—	—	—

As previously stated, the sera of a number of dogs reacted positively with the extracts of parasites the ova of which could not be found in the feces. Whether the antibody persisted in the body fluids after the parasite had been expelled, or whether we failed to find the ova altho the parasite was present, we are unable to decide. A third

possibility of much importance is the question of pseudo, or false, positive and non-specific reactions. All tests were conducted with one-quarter of the anticomplementary dose of the antigen in each case, and in all tests in which the serum control-tube showed an anticomplementary state of the serum, the results have been excluded. The results of complement-fixation tests in this series of dogs are shown in Table 5.

TABLE 5

RESULTS OF COMPLEMENT-FIXATION TESTS WITH SERA OF DOGS SHOWING NO OVA IN FECES

Dog	Serum	Dose Serum, c.c.	Salt-Solution Extract T. Ser-rata	Alcoholic Extract T. Ser-rata	Salt-Solution Extract Dip-ylidium Can-ninum	Alcoholic Extract Dip-ylidium Can-ninum	Salt-Solution Extract As-caris Can-is	Alcoholic Extract As-caris Can-is	Salt-Solution Extract Tri-cho-cephalus Dis-par	Salt-Solution Extract Stron-gylus Gigas	Alcoholic Extract T. Sagi-nata	Serum Control
224A	Active	0.1	—	++++	—	—	+	—	—	—	—	—
235A	Active	0.1	+	++++	—	++	—	—	+++	+++	—	—
242A	Active	0.1	—	++++	—	—	++	—	—	—	—	—
328	Active	0.1	—	—	—	—	—	—	—	—	—	—
333	Active	0.1	—	—	—	—	—	—	—	—	—	—
246A	Active	0.1	—	—	—	—	—	—	—	—	—	—
247A	Active	0.1	++	++++	—	—	—	—	—	—	—	—
285	Active	0.1	—	—	—	—	—	—	—	—	—	—
290	Active	0.1	—	—	—	—	—	+	—	—	—	—
298	Active	0.1	—	—	—	—	—	—	—	—	—	—
324	Active	0.1	—	—	—	—	—	—	—	—	—	—
325	Active	0.1	—	—	—	—	—	—	—	—	—	—
333	Active	0.1	—	—	—	—	—	—	—	—	—	—
390	Active	0.1	—	—	—	—	—	—	0	—	—	—
399	Active	0.1	—	—	—	—	—	—	0	—	—	—
493	Active	0.1	—	—	—	++++	++	++	—	—	—	—
448	Active	0.2	—	—	—	—	—	—	—	—	—	—
451	Active	0.2	—	—	—	—	—	—	—	—	—	—
455	Active	0.2	—	—	—	—	—	—	—	—	—	—
449	Active	0.2	—	—	—	—	—	—	—	—	—	—
461	Active	0.2	—	—	—	—	+	—	—	—	—	—
476	Active	0.2	—	—	—	—	—	—	—	—	—	—
463A	Inactive	0.1	++++	++++	—	—	+++	—	—	—	—	—
337	Inactive	0.1	++++	++++	—	—	—	—	—	—	—	—
431	Inactive	0.1	+	++++	—	—	—	+	—	—	—	—
433	Inactive	0.1	++	++++	—	—	—	+	—	—	+	—
435	Inactive	0.1	+++	++++	+	—	—	±	—	—	—	—
436	Inactive	0.1	++++	++++	—	+	—	—	—	—	—	—

Particular attention is drawn to the results of the complement-fixation tests with the inactivated sera. These were heated at 56 C. for one-half hour. As emphasized in our previous studies in non-specific complement fixation with normal rabbit and dog sera, heating sera at 56 C. greatly increases the antilytic activity and the percentage of non-specific complement-fixations. For this reason the majority of our tests have been conducted with fresh, active sera in dose of 0.1 c.c.

Heating at 62 C. tends to remove a large amount of the non-specific antilytic and complement-absorbing substances without materially depreciating the antibodies, and, when heating is necessary on account of thermolabil anticomplementary substances in serum, this degree of heat should be used.

TABLE 6  
SUMMARY OF THE RESULTS OF COMPLEMENT-FIXATION TESTS WITH DOG SERA AND EXTRACTS OF VARIOUS PARASITES

Antigen	Number of Dogs Tested	Reactions		Percentage Positive
		Positive	Negative	
Salt-solution extract of <i>T. serrata</i> .....	100	20	80	20
Alcoholic extract of <i>T. serrata</i> .....	100	42	58	42
Salt-solution extract <i>Dipylidium caninum</i> .....	100	4	96	4
Alcoholic extract of <i>Dipylidium caninum</i> .....	100	11	89	11
Salt-solution extract of <i>Ascaris canis</i> .....	100	17	83	17
Alcoholic extract of <i>Ascaris canis</i> .....	100	7	93	7
Salt-solution extract of <i>Trichocephalus dispar</i> (whip worm) .....	91	3	88	3.3
Salt-solution extract of <i>Strongylus gigas</i> .....	98	4	94	4
Alcoholic extract of <i>T. saginata</i> .....	99	2	97	2

As will be noted in Tables 2 to 4, many of the sera reacted positively with antigens of parasites the ova of which were not found in the feces. These results are summarized in Table 6, showing the percentage of positive reactions observed with the various antigens irrespective of whether the ova were or were not found in the feces.

In general the alcoholic extracts yielded a higher percentage of positive reactions than did the salt-solution extracts.

TABLE 7  
COMPARISON OF RESULTS OF POSITIVE COMPLEMENT-FIXATION TESTS WITH THOSE OF POSITIVE FECES EXAMINATIONS

Parasite	Percentage Positive Reactions		Percentage Positive Feces Examinations
	Salt-Solution Extracts	Alcoholic Extracts	
<i>Tenia serrata</i> .....	20	42	6
<i>Dipylidium caninum</i> .....	4	11	3.6
<i>Ascaris canis</i> .....	17	7	40
<i>Trichocephalus dispar</i> .....	3.3	0	43

Based on the examination of the feces of 100 dogs the following comparison has been made of the percentage of positive complement-fixations with the percentage of positive examinations for ova (Table 7). According to our results, it would appear that production of antibodies is most likely in infestations with the tapeworms (*T.*

serrata and *Dipylidium caninum*). Antibodies are also produced in infestations with *Ascaris canis* and the whip-worm, but in less degree; particularly is this true in infestations with the whip-worm.

TABLE 8

RESULTS OF COMPLEMENT-FIXATION TESTS WITH ACTIVE SERA OF DOGS SHOWING PARASITIC OVA IN THE FECES

Doses of Serum, c.c.	Antigens				Serum Control
	Salt Solution T. Serrata	Salt Solution Ascaris Canis	Alcoholic Extract Ascaris Canis	Salt Solution Dipylidium Caninum	
Dog 486.—Ascaris Canis and Ankylostoma Caninum					
0.01.....	—	±	—	—	—
0.05.....	—	++	—	—	—
0.1.....	—	+++	+	—	—
0.2.....	—	+++	++	—	—
Dog 502.—Ascaris Canis and Trichocephalus Dispar					
0.01.....	—	—	—	—	—
0.05.....	—	—	—	—	—
0.1.....	—	—	—	—	—
0.2.....	—	—	—	—	—
Dog 505.—Ascaris Canis and Trichocephalus Dispar in Feces					
0.01.....	—	—	—	—	—
0.05.....	—	—	—	—	—
0.1.....	—	—	—	—	—
0.2.....	—	—	—	—	—
Dog 528.—Ascaris Canis in Feces					
0.01.....	—	±	—	—	—
0.05.....	—	++	—	—	—
0.1.....	—	++++	+	—	—
0.2.....	—	++++	++	—	—
Dog 319.—H. Serrata and Ascaris Canis in Feces					
0.01.....	±	—	—	±	—
0.05.....	++	+	—	+	—
0.1.....	+++	++	—	++	—
0.2.....	++++	++	+	++	—

## SPECIFICITY OF THE COMPLEMENT-FIXATION

In view of the observation that the sera of dogs showing the presence of the ova of one parasite in the feces not infrequently reacted

positively with the antigens of other parasites, it is possible that the antibodies are not highly specific, and that in the dose of serum used (0.1 c.c.) the antibody for one parasite reacted with the extracts of other parasites in a general manner, as is not infrequently observed in complement-fixation with the immune sera against closely related micro-organisms such as the streptococci, diphtheria group, etc. We have studied this phase of the subject by conducting complement-fixation tests with descending doses of serum, and also by immunizing rabbits with the salt-solution extracts of various parasites and testing the specificity of the antibodies in a series of cross complement-fixation experiments.

*Complement-fixation Tests with Descending Doses of Sera.*—The results of 5 experiments with descending doses of the sera of 5 infested dogs with several antigens are given in Table 8. Ten such experiments were conducted, of which those given are examples of the results observed.

TABLE 9  
RESULTS OF COMPLEMENT-FIXATION TESTS WITH THE INACTIVATED SERUM OF DOG 24 SHOWING  
THE OVA OF *T. SERRATA* IN THE FECES

Doses of Serum c.c.	Antigens					Serum Control
	Salt Solution <i>T.</i> <i>Serrata</i>	Alcoholic Extract <i>T.</i> <i>Serrata</i>	Alcoholic Extract <i>Ascaris</i> <i>Canis</i>	Alcoholic Extract of <i>Syphilitic</i> Liver	Cholester- inized Extract of Heart	
0.01	—	±	±	—	±	—
0.05	+	++	+	+	++	—
0.1	++	+++	+	++	++++	—
0.2	+++	++++	+++	++	++++	±

The experiments tabulated in Table 8 show that the antibody for *Ascaris canis* does not absorb complement with antigens of *T. serrata* and *Dipylidium caninum*. The antibody for *T. serrata*, however, absorbed complement in some degree with the antigen of *Dipylidium caninum*, and it is an open question as to whether these results may indicate a biologic relation between these parasites. The antibodies, therefore, when shown in the serum, appear to be highly specific, in so far, at least, as between those produced against *Ascaris canis* and those against *T. serrata* or *Dipylidium caninum*.

When inactivated sera (i. e. heated at 56 C.) are employed, and especially those of dogs showing well-marked tendencies toward non-specific complement fixation, specificity is poorly marked, or entirely lost, as shown in Table 9.

*Cross Complement-fixation Tests With Immune Sera.*—A series of rabbits were immunized by repeated intravenous injections of salt-solution extracts of the following parasites: *T. serrata*, *Dipylidium caninum*, *Ascaris canis*, *Trichocephalus dispar*, *Strongylus gigas*.

TABLE 10  
RESULTS OF CROSS COMPLEMENT-FIXATION TESTS WITH VARIOUS IMMUNE SERA (ACTIVE)

Active Serum, c.c.	Antigens					Serum Control
	Salt Solution <i>T. Serrata</i>	Salt Solution <i>Dipylidium Caninum</i>	Salt Solution <i>Ascaris Canis</i>	Salt Solution <i>Trichocephalus Dispar</i>	Salt Solution <i>Strongylus Gigas</i>	
With <i>Tenia Serrata</i> Immune Serum						
0.001	+++	—	—	—	—	—
0.005	++++	+	—	—	—	—
0.01	++++	+++	—	—	—	—
0.05	++++	++++	—	—	+	—
0.1	++++	++++	+	+	+	—
0.2	++++	++++	++	++	++	—
With <i>Dipylidium Caninum</i> Immune Serum						
0.001	—	—	—	—	—	—
0.005	+	+	—	—	—	—
0.01	+++	++	—	—	—	—
0.05	++++	+++	+	—	+	—
0.1	++++	++++	++	+	++	—
0.2	++++	++++	+++	++	+++	—
With <i>Ascaris Canis</i> Immune Serum						
0.001	—	—	—	—	—	—
0.005	—	—	+	—	—	—
0.01	—	—	++	—	—	—
0.05	—	—	++++	—	+	—
0.1	+	—	++++	—	++	—
0.2	+++	—	++++	—	+++	—
With <i>Trichocephalus Dispar</i> Immune Serum						
0.001	—	—	—	±	—	—
0.005	—	—	—	+	—	—
0.01	—	—	—	++++	—	—
0.05	—	—	+	++++	++	—
0.1	++	—	++	++++	++	—
0.2	+++	—	+++	++++	+++	—
With <i>Strongylus Gigas</i> Immune Serum						
0.001	—	—	—	—	++	—
0.005	—	—	—	—	++++	—
0.01	—	—	+	—	++++	—
0.05	—	—	++	—	++++	—
0.1	—	—	++++	—	++++	—
0.2	+	+	++++	—	++++	—

The material for these injections was prepared in the same manner as the salt-solution extracts used as antigens, except that they were preserved with 0.25% phenol instead of 0.5%.

Each rabbit received 6 injections at intervals of 5 days of 1, 2, 2, 3, 3, and 5 c.c., respectively, of each extract. One week after the last dose the animal was bled, the serum separated, and the tests conducted on the same and following days.

TABLE 11  
RESULTS OF CROSS COMPLEMENT-FIXATION EXPERIMENTS WITH VARIOUS IMMUNE SERA  
(HEATED AT 62 C.)

Heated Serum, c.c.	Antigens					Serum Control
	Salt Solution T. Serrata	Salt Solution Dipylidium Caninum	Salt Solution Ascaris Canis	Salt Solution Trichocephalus Dispar	Salt Solution Strongylus Gigas	
With T. Serrata Immune Serum						
0.001	+	—	—	—	—	—
0.005	++++	—	—	—	—	—
0.01	++++	+	—	—	—	—
0.05	++++	++	—	—	—	—
0.1	++++	+++	—	—	+	—
0.2	++++	+++	—	+	++	—
With Ascaris Canis Immune Serum						
0.001	—	—	—	—	—	—
0.005	—	—	±	—	—	—
0.01	—	—	+	—	+	—
0.05	—	—	++++	—	++	—
0.1	—	—	+++++	—	+++	—
0.2	—	—	+++++	—	++++	—
With Trichocephalus Dispar Immune Serum						
0.001	—	—	—	—	—	—
0.005	—	—	—	+	—	—
0.01	—	—	—	++	—	—
0.05	—	—	+	+++	—	—
0.1	+	—	+	++++	+	—
0.2	++	—	++	++++	+++	—
With Strongylus Gigas Immune Serum						
0.001	—	—	—	—	+++	—
0.005	—	—	—	—	++++	—
0.01	—	—	+	—	++++	—
0.05	—	—	+++	—	++++	—
0.1	—	—	+++++	—	++++	—
0.2	—	—	+++++	—	++++	—

All antigens were titrated before each experiment and used in one-quarter of their anticomplementary doses, as previously described.

These cross complement-fixation tests were conducted with fresh, active sera, sera heated at 56 C. for 30 minutes, and again with sera heated at 62 C. for 30 minutes.



The results observed with fresh, active sera are shown in Table 10; with sera heated at 62 C., in Table 11. They may be summarized as follows:

1. The active immune serum of *T. serrata* showed complement-absorption in best degree with its homologous antigen, and also to a well-marked extent with the antigen of *Dipylidium caninum* (Table 10). As shown in Table 8, similar reactions were observed with the serum of a dog infested with *T. serrata*, and in view of the wide morphologic differences between these tape-worms, we leave it an open question as to whether these reactions may be interpreted as indicating a biologic relation between them.

This relationship is again shown in Table 10, in the immune serum of *Dipylidium caninum*, which absorbed complement with the extract of *T. serrata* to the same degree as with its own or homologous antigen.

2. The active immune serum of *Ascaris canis* showed complement-absorption in best degree with its homologous antigen and to some extent with the antigen of *Strongylus gigas* (Table 10). These reactions may be interpreted as showing a biologic relation between these parasites, and this probable relationship is further indicated by the results observed with the immune serum against *Strongylus gigas*, which absorbed complement best with its own antigen and to a well-marked degree with the antigen against *Ascaris canis* (Table 10).

3. As shown in the tables, slight reactions frequently occurred with other antigens with the lower doses of serum. These reactions may have been due to non-specific complement fixation. Unfortunately, none of the rabbits used was subjected to preliminary complement-fixation tests before being accepted for the purposes of immunization, and we now believe that this precaution is of much importance when rabbit immune sera are being used in complement-fixation tests. Only those rabbits the sera of which are known to be free of non-specific complement-fixation bodies by reason of negative results in one or more preliminary complement-fixation tests should be selected for purposes of immunization. It is probable that the sera of rabbits used in preparing the immune sera shown in Table 10 would have shown non-specific complement fixation in doses of 0.1 and 0.2 c.c. before immunization.

4. Since heating rabbit and dog sera<sup>18</sup> at 62 C. tends to remove a portion or all of the non-specific complement-absorbing substances, we

<sup>18</sup> Kolmer: Jour. Infect. Dis., 1916, 18, pp. 20, 27.

repeated these cross complement-fixation tests with sera heated at this temperature for half an hour. The results are shown in Table 11.

In this series of experiments the same biologic relation is indicated between *T. serrata* and *Dipylidium caninum* and between *Ascaris canis* and *Strongylus gigas*, while the reactions between the immune sera and other heterologous antigens are decreased or removed entirely as a result of heating the sera at 62 C.

On the other hand, heating these immune sera at 56 C. increased to an appreciable extent the tendency toward non-specific complement fixation. Accordingly, it would appear that complement-fixation tests with rabbit sera should be conducted with fresh, active sera, or, if heating is necessary to remove thermolabile anticomplementary bodies, with sera inactivated at 62 C. rather than at 56 C.

Of interest in this connection from the standpoint of applying results in complement-fixation tests in the diagnosis of intestinal parasitism of persons, is the question regarding the possibility of the sera of syphilitic persons reacting with the extracts of intestinal parasites. We have conducted a number of complement-fixation tests with luetic sera and the extracts of parasites and found that some complement fixation occurred, especially with the alcoholic extracts. These results are not surprising in view of the fact that the extracts contained lipoidal substances which were capable of absorbing complement with the syphilis reagin, as are other lipoidal substances.

We have tested the sera of 2 persons infested with *T. saginata* with these extracts of parasites from dogs, as well as with salt-solution and alcoholic extracts of *T. saginata*. The serum of one of these patients yielded strongly positive reactions with the extracts of *T. saginata* and *T. serrata* and to some extent with *Dipylidium caninum*, but none with the other antigens. The serum of the second person failed to fix complement with all antigens.

So far we have not had the opportunity of testing the serum of a syphilitic person infested with an intestinal parasite. It is probable, however, that if antibodies for both syphilis and parasite were present, these could be distinguished by a complement-fixation test conducted with constant doses of antigen and descending doses of serum, in which the group reaction would be lost in the smaller doses as 0.001, 0.005, 0.01, and 0.05 c.c. serum.

#### CONCLUSIONS

The results of this study are summarized in several subdivisions throughout the paper; here it may be stated that according to the results

of complement-fixation tests with the sera of infested dogs we have reason to believe that production of antibodies may occur after infestation of the intestines with the common parasites.

Production of antibodies was especially in evidence in infestations with tapeworms; to a less degree with the ascarides or round worms, and to a slight extent with the whip-worm.

These complement-fixations have tended to show a biologic relation between the tapeworms *Tenia serrata* and *Dipylidium caninum* and between *Ascaris canis* and *Strongylus gigas*, altho on account of the wide morphologic differences we leave it an open question; it is probable, therefore, that complement-fixation tests will not differentiate with the usual technic between related species of parasites, altho they may show the presence of a parasite.

Complement-fixation tests may be of value in the diagnosis of intestinal parasitism of man, and we are now making investigations in this field.